study demonstrating that some HIV-1 protease inhibitors (PIs) inhibit the growth of *Plasmodium falciparum* in vitro [2]. Their findings on the in vivo activity of HIV-1 PIs in a murine model are in accordance with the results of our own studies (K. T. Andrews, D. P. Fairlie, P. K. Madala, J. Ray, P. M. Hilton, L. A. Melville, L. Beattie, T. Skinner-Adams, R. C. Reid, M. J. Stoermer, D. Gardiner, and J. S. McCarthy, unpublished data) and support our hypothesis [2] that the antimalarial activity of these drugs may be clinically relevant, particularly for those individuals with HIV-1 and malarial coinfections.

We were unaware of the hypotheses and the bioinformatic analyses published by Savarino et al. in March 2004 [3]. Although our in vitro study was published in the 1 December 2004 issue of the *Journal of Infectious Diseases,* it was submitted for publication in April of the same year. We do, however, agree with their comments on the structural similarities between *P. falciparum* plasmepsin II and IV and the HIV-1 aspartic protease. Since our initial in vitro study, we have conducted more-detailed modeling studies of the possible interactions between the plasmepsins and the HIV-1 PIs. These in silico comparisons have extended beyond the whole-protein sequence alignment that was discussed in our initial article and have entailed study of the 3-dimensional crystal structures of both enzymes with inhibitors. Our unpublished observations concur with the findings of Savarino et al. and demonstrate that both plasmepsin II and IV are similar to the HIV-1 protease with respect to structure and active site. Interestingly, inhibitors of both plasmepsin II (including Rs370, which has been cocrystallized with plasmepsin II [4]) and HIV-1 display similar structural characteristics and can be docked into the active sites of both proteases (figure 1). Although our in silico data and the comparisons described by Savarino et al. suggest that the HIV-1 PIs may inhibit the growth of *Plasmodium* parasites by block-ing the action of a plasmepsin, it is important to remember that all digestive vacuole *P. falciparum* plasmepsins can be knocked out; the removal of each single enzyme alone is not lethal to parasites in vitro [6, 7]. Thus, if the plasmepsins are the target of HIV-1 PIs, they probably target >1 enzyme. Also, at this point, alternative methods of action cannot be ruled out.

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Provirus Load Is Lower in Human T Lymphotropic Virus (HTLV)–II Carriers Than in HTLV-I Carriers: A Key Difference in Viral Pathogenesis?

To the Editor—Human T lymphotropic virus (HTLV)–I and HTLV-II are closely related retroviruses [1]. In addition to HTLV-I–associated myelopathy (HAM), HTLV-I is associated with adult T cell leukemia (ATL). Some evidence suggests that HTLV-II may be associated with a HAM-like neuropathy [2], but the incidence of HTLV-II–associated neuropathy is low and its association with any malignancy is uncertain. Why the pathogenesis of HTLV-II disease differs from that of HTLV-I disease—despite high homologies of viral sequences—is unclear.

Provirus load, the amount of integrated virus in the host genome, is a correlate of HTLV-I disease pathogenesis. HTLV-I provirus loads are higher in patients with ATL or HAM than in asymptomatic carriers [1, 3]. Higher provirus load occurs because of greater viral replication and proliferation of HTLV-I–infected clones. Although the association between HTLV-II provirus load and disease pathogenesis is less well studied, a correlation between provirus load and clonal proliferation of HTLV-II–infected cells has been observed [4].

In the 1 August 2004 issue of the *Journal of Infectious Diseases,* Murphy et al. [5] reported that HTLV-I provirus load was higher than HTLV-II provirus load in US blood donors. In the study, HTLV-II provirus load was found to be unrelated to age but was higher in men than in women. In this letter, we confirm the finding of a significant difference in provirus load between HTLV-I and HTLV-II carriers.

Between 1983 and 1990, we recruited injection drug users from methadone maintenance treatment centers in 3 US metropolitan areas in New York, New Jersey, and Louisiana [6]. Cyropreserved peripheral-blood mononuclear cells (PBMCs) were available from 11 HTLV-I and 91 HTLV-II carriers, all of whom were HIV-1 negative. HTLV-I and HTLV-II provirus

Table 1. Sex, age, provirus detection, and provirus load (mean, median, and range) in 102 US injection drug users, by human T lymphotropic virus (HTLV) type.

Characteristic	HTLV-II	HTLV-I	P
No. of subjects	91	11	
No. (%) of men	59 (64.8)	8 (72.7)	.37
Age, years			
Mean	44.5	43.8	.91[a]
25th percentile	36.8	34.8	
50th percentile	42.8	43.5	
75th percentile	49.0	46.9	
CD4-positive cells, %			
Mean	38.6	39.5	.61[a]
25th percentile	34.5	30.2	
50th percentile	38.6	40.2	
75th percentile	44.3	42.7	
HTLV provirus detection, no. (%) of subjects	62 (68.1)	9 (81.8)	.50
HTLV provirus load, $\log_{10}$ copies/1×10^5 PBMCs[b]			
Mean	2.43	3.43	.01[a]
25th percentile	0.70	0.70	
50th percentile	2.60	3.74	
75th percentile	3.69	4.34	

NOTE. PBMCs, peripheral-blood mononuclear cells.
[a] P values compare the means of continuous variables between the HTLV-I and HTLV-II carriers.
[b] Mean and percentile provirus loads include both detectable and undetectable values.

loads were quantitated by use of a real-time polymerase chain reaction (PCR) assay, with sequences from the *tax* gene used as a primer. This assay detects HTLV-I and HTLV-II provirus loads with equal sensitivity. For each sample, ~1 μg of DNA was amplified for 45 cycles by use of AmpliTaq Gold polymerase with an ABI PRISM Sequence Detection System and TaqMan PCR Reagent (PE Applied Biosystems) [7]. Undetectable provirus load was assigned a value of 5 copies/1×10^5 PBMCs, the midpoint between 0 and the detection limit. The $\log_{10}$-transformed provirus loads in the HTLV-I and HTLV-II carriers were compared by the Kruskal-Wallis test. Age, sex, percentage of CD4-positive cells (hereafter, "CD4 percent"), and provirus detection were compared either by the χ^2 test or by Fisher's exact test.

The mean age and CD4 percent at the time of blood collection did not differ between the HTLV-I and HTLV-II carriers (table 1). Twenty-nine (31.9%) of the 91 HTLV-II carriers had undetectable provirus loads, compared with 2 (18.2%) of the 11 HTLV-I carriers (P = .50). The mean $\log_{10}$ provirus load was lower in the 91 HTLV-II carriers than in the 11 HTLV-I carriers (2.43 vs. 3.43 $\log_{10}$ copies/1×10^5 PBMCs; P = .01). The HTLV-I provirus load reported in our study, which corresponds to 2.7% of lymphocytes being infected, is consistent with what has been reported in asymptomatic HTLV-I carriers in other cohorts [8, 9] but is somewhat higher than the mean reported by Murphy et al. (3.28 $\log_{10}$ copies/1×10^6 PBMCs, which corresponds to 0.19% of lymphocytes being infected).

In contrast to Murphy et al.'s findings, HTLV-II provirus load was not significantly different in 32 men and 59 women in our cohort (2.31 vs. 2.65 $\log_{10}$ copies/1×10^5 PBMCs; P = .30). In a linear regression model in which sex was adjusted for, the mean HTLV-II provirus load decreased by 0.33 $\log_{10}$ copies/1×10^5 PBMCs (95% confidence interval, 0.01–0.65 $\log_{10}$ copies/1×10^5 PBMCs) per every 10-year increment in age (P = .04). HTLV-I provirus load was also similar in 8 men and 3 women (3.31 vs. 3.77 $\log_{10}$ copies/1×10^5 PBMCs; P = .68), but the small number of subjects limited our ability to

further analyze the associations between HTLV-I provirus load and CD4 percent and age.

The discrepancies in the results of the 2 studies could be due to many factors, including differences in the characteristics of the study populations, such as route of infection and drug-use practices. Although the mean ages of the HTLV-II carriers in each study were similar, our population consisted entirely of injection drug users, whereas Murphy et al.'s population included subjects who did not use injection drugs. An association between older age and lower HTLV-II provirus load in our population is of interest. For HTLV-I carriers, HAM incidence appears to peak between 40 and 50 years of age and decreases thereafter. The age-specific incidence of HAM-like neuropathy in HTLV-II carriers has not been established.

In summary, the results of these 2 studies confirm that a finding of a higher provirus load in HTLV-I carriers than in HTLV-II carriers is not limited to a specific study population and is not a result of varying sensitivities of PCR assays. These findings support the possibility that a lower disease incidence in HTLV-II carriers than in HTLV-I carriers may be due to lower levels of HTLV-II provirus load.

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Reply to Hisada et al.

To the Editor—Hisada et al. [1] provide interesting data that support the major finding of the recently published study [2] by my colleagues and me but that do not support some minor findings. Both studies analyzed cross-sectional data from prevalent cohorts and found that proviral load was up to 1 log$_{10}$ higher in human T lymphotropic virus (HTLV)–I carriers than in HTLV-II carriers. Replication of this finding strengthens the conclusion that the magnitude of proviral load may explain the differences in pathogenesis between the 2 retroviruses—differences that exist despite the similar genetic sequences of the viruses. It may also explain why both serologic and nucleic-acid assays are less sensitive for HTLV-II than for HTLV-I, a matter that has been only partially addressed by the blood bank community [3–5]. It would be interesting
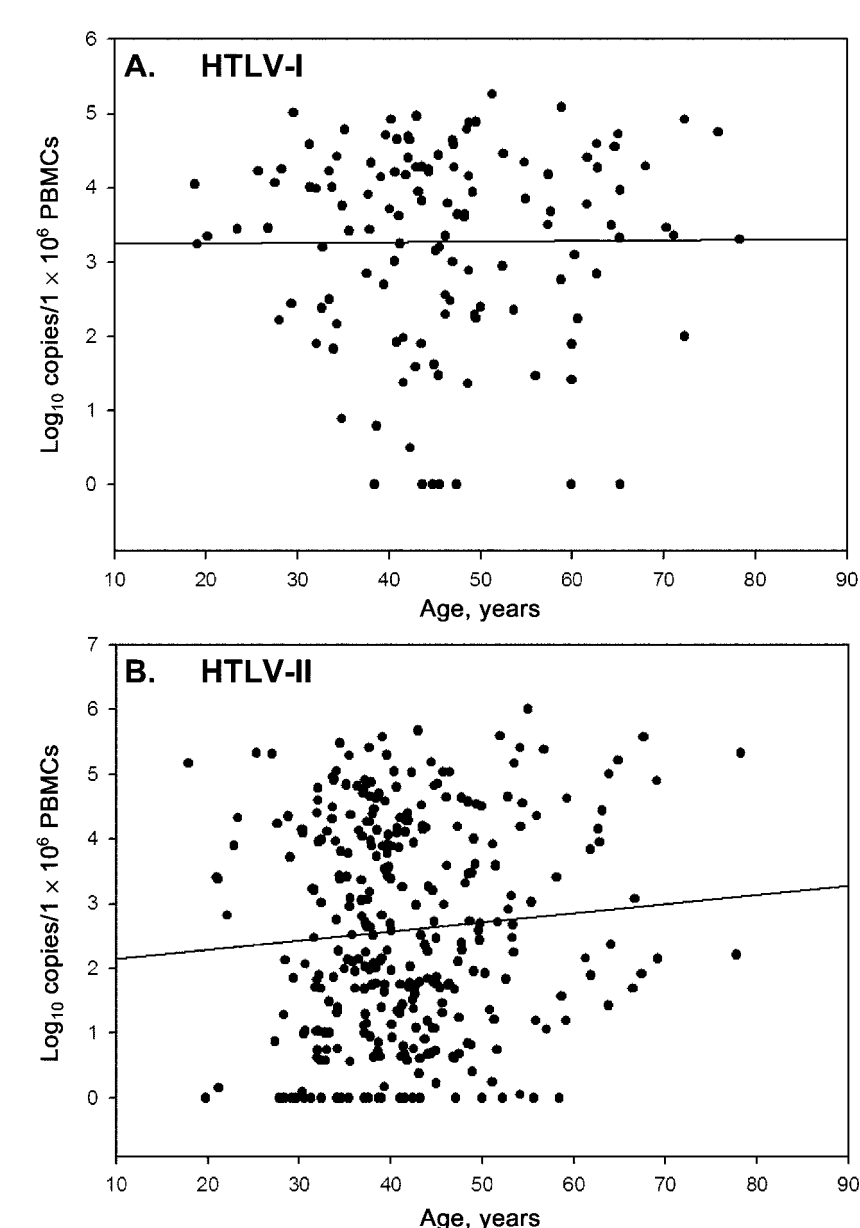

Figure 1. *A,* Human T lymphotropic virus (HTLV)–I proviral load, by age, in baseline samples from 127 HTLV-I–infected subjects in the HTLV Outcomes Study ($\beta = .001$; $P = .92$). *B,* HTLV-II proviral load, by age, in baseline samples from 328 HTLV-II–infected subjects in the HTLV Outcomes Study ($\beta = .014$; $P = .14$). PBMCs, peripheral-blood mononuclear cells.

if Hisada et al. could perform HTLV-II subtyping for their cohort, to test the finding reported by my colleagues and me of a higher proviral load in persons infected with HTLV-II subtype A than in persons infected with HTLV-II subtype B.

Despite the use by both studies of primers directed at *tax* gene sequences that are conserved between HTLV-I and HTLV-II, the ~1 log$_{10}$ higher mean proviral loads in

Hisada et al.'s study (7-fold for the HTLV-II carriers and 14-fold for the HTLV-I carriers) are most likely the result of technical differences in the quantitative polymerase chain reaction assays used. Testing of shared reagents and reference standards by these and other laboratories, as proposed recently during a workshop at the 11th International Conference on Human Retrovirology: HTLV and Related Viruses